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Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines

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ABSTRACT. Using only one type of marker to quantify genetic diversity generates results that have been questioned in terms of reliability, when compared to the combined use of different markers. To compare the efficiency of the use of single versus multiple markers, we quantified genetic diversity among 10 S_7 inbred popcorn lines using both RAPD and SSR markers, and we evaluated how well these two types of markers discriminated the popcorn genotypes. These popcorn genotypes: "Yellow Pearl Popcorn" (P1-1 and P1-5), "Zélia" (P1-2 and P1-4), "Curagua" (P1-3), "IAC 112" (P9-1 and P9-2), "Avati Pichinga" (P9-3 and P9-5), and "Pisankalla" (P9-4) have different soil and climate adaptations. Using RAPD marker analysis, each primer yielded bands of variable intensities that were easily detected, as well as non-specific bands, which were discarded from the analysis. The nine primers used yielded 126 bands, of which 104 were classified as polymorphic, giving an average of 11.6 polymorphisms per primer. Using SSR procedures, the number of alleles per locus ranged from two to five, giving a total of 47 alleles for the 14 *SSR* loci. When comparing the groups formed using SSR and RAPD markers, there were similarities in the combinations of genotypes from the same genealogy. Correlation between genetic distances obtained through RAPD and SSR markers was relatively high (0.5453), indicating that both techniques are efficient for evaluating genetic diversity in the genotypes of

popcorn that we evaluated, though RAPDs yielded more polymorphisms.

Key words: *Zea mays*; DNA markers; RAPD and *SSR* loci; Microsatellites; Comparative analysis of molecular groups

INTRODUCTION

Maximum potential for genetic gain is proportional to phenotypic variation (σf) present in the original population and maintained in subsequent selection cycles. Phenotypic variation is positively associated with genetic diversity, but is also dependent on environmental factors as well as on the interaction between genotypes and environment (Moose and Mumm, 2008). Thus, determining genetic diversity through variation between genotypes, genotype groups, or populations is essential to plant genetic breeding programs. These measures are all important for the identification of genetically distant parental combinations, aiming to use distinct gene sets in crossings for superior hybrids and segregants, to evaluate the degree of genetic erosion, or even to determine the extent of the genetic base of cultivated forms to develop heterotic groups (Amaral Júnior, 1999a,b; Dandolini et al., 2008; Gonçalves et al., 2008a; Munhoz et al., 2009).

Determining genetic diversity can be based on agronomic, morphological, biochemical, and molecular types of information, among others (Mohammadi and Prasanna, 2003; Sudré et al., 2007; Gonçalves et al., 2009). However, molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level and without interferences from environmental factors, and where they involve techniques that provide fast results detailing genetic diversity (Binneck et al., 2002; Garcia et al., 2004; Saker et al., 2005; Gonçalves et al., 2008b; Souza et al., 2008).

With the beginning of studies that led to the development of polymerase chain reaction (PCR) technology (Saiki et al., 1985; Mullis and Faloona, 1987), there were amazing advances in the refinement of techniques to obtain specific or non-specific DNA fragments, relevant mainly to research in genetic diversity. The following techniques are those most used and listed in chronological order: SSR (simple sequence repeats or just microsatellites) (Tautz, 1989), RAPD (randomly amplified polymorphic DNA) (Williams et al., 1990) or AP-PCR (arbitrarily primed PCR) (Welsh and McClelland, 1990), ISSR (inter-simple sequence repeats) (Zietkiewicz et al., 1994), AFLP (amplified fragment length polymorphism) (Vos et al., 1995), SNPs (single nucleotide polymorphisms) (Chen and Sullivan, 2003) and, more recently, DarT (diversity array technology) (Kilian et al., 2005). These different types of molecular markers are also different as to their potential to detect differences between individuals, their cost, facilities required, and consistency and replication of results (Schlötterer, 2004; Schulman, 2007; Bernardo, 2008).

As to popcorn, research in genetic diversity is of great importance to genetic breeding programs, due to the narrow genetic base of the crop, which can have its origin in selections of the common maize of the flint kind (Ziegler and Ashman, 1994). In this sense, some researchs using molecular markers have been carried out, aiming at a better understanding of the diversity and parentage among popcorn varieties, such as those using ISSR (Kantety et al., 1995), RAPD (Vilela et al., 2008), and SSR (Li et al., 2004; Santacruz-Varela et al., 2004; Aguiar et al., 2008; Dandolini et al., 2008; Bracco et al., 2009; Munhoz et al., 2009; Silva et al., 2009).

However, the combined use of different markers can provide more reliable information about genetic diversity when compared to the use of only one marker. The expectation is that some errors or problems presented by a certain marker could be minimized using other markers (Demeke et al., 1997; Saker et al., 2005; Souza et al., 2008). Souza et al. (2008), comparing

genetic diversity between maize through the use of RAPD and SSR markers, found that RAPD markers were effective in evaluating the genealogy of materials, while SSR was essential to recognize differences between quantitative characteristics.

Thus, the objectives of this study were: i) to estimate and characterize genetic divergence among 10 popcorn lines from plants originating from different soil and climate adaptations, using RAPD and SSR markers, and ii) to evaluate, in a comparative way, the consistency of the information obtained using RAPD and SSR markers.

MATERIAL AND METHODS

Plant materials and DNA extraction

Ten S_7 popcorn inbred lines were used from the genotypes "Yellow Pearl Popcorn" (P1-1 and P1-5), "Zélia" (P1-2 and P1-4), "Curagua" (P1-3), "IAC 112" (P9-1 and P9-2), "Avati Pichinga" (P9-3 and P9-5), and "Pisankalla" (P9-4). These lines were developed at the Experimental Farm of Maringá State University (Maringá, PR, Brazil).

Total cellular DNA was extracted from the young leaves of the lines using the methodology described by Hoisington et al. (1994). After DNA extraction, DNA quantity and quality were determined by 0.8% agarose gel electrophoresis. The marker used was a standard DNA solution (λ phage) of gradual and known concentrations (50, 100 and 150 ng). The gel was stained with 0.5 µg/mL ethidium bromide and the image was visualized with a High-Performance Ultraviolet Transilluminator - Edas 290, using the 1-D 3.5 Kodak program.

RAPD analysis

In order to obtain RAPD fragments, an initial selection of primers was performed. Therefore, the A (1 to 20), B (1 to 20), C (1 to 20), F (5, 9 and 13), L (11), M (1 to 10), and P (2, 4, 7, 8, 9, 10, 11, and 17) kits of Operon Technologies Inc., Alameda, CA, USA, were used. Of the 83 primers, nine amplified comprising a standard of well-defined bands, and they were selected to quantify genetic diversity among the popcorn lines. The primers selected were: OPA03, OPA12, OPB07, OPB17, OPC05, OPC11, OPF05, OPL11, and OPM02. In order to standardize the DNA quantities to be used in the RAPD amplification reactions, 10, 15, 20, 25, and 30 ng DNA were tested. For the amplifications, the concentration of $MgCl_2$ was also standardized by first testing concentrations of 2.0, 2.5, 3.0, and 3.5 mM.

Amplification reactions were carried out in a Techne TC-512 thermocycler, according to Williams et al. (1990). DNA denaturation was performed at 96°C for 5 min. This step was followed by 45 amplification cycles (94°C for 1 min, 35° for 1 min, 72° for 2 min). After the 45 cycles, a final extension of 7 min at 72°C was performed. Amplification products were separated on a 1.7% agarose gel. The molecular weight marker used in the gels was 1-kb DNA ladder (Invitrogen). Gels were stained with 0.5 µg/mL ethidium bromide, and the image was visualized with a High-Performance Ultraviolet Transilluminator - Edas 290, using the 1-D 3.5 Kodak program.

SSR analysis

As to the amplification reactions using the SSR markers, 51 primers were selected, obtained at http://www.maizegdb.org/ssr.php. Of these primers, 14 were selected, due to the fact that they

provided greater complementarity and reproducibility, as well as the presence of polymorphism. Amplification reactions were performed in a Techne TC-512 thermocycler, using the Touchdown PCR program (Don et al., 1991). Amplified fragments were separated by 4% agarose gel electrophoresis (50% normal agarose and 50% MS-8 agarose). In order to estimate the size of the amplified fragments, a 100-bp molecular weight marker (Invitrogen) was used. Gels were run at 60 V for 5 h. Afterward, gels were stained with 0.5 µg/mL ethidium bromide, and the image was revealed with a High-Performance Ultraviolet Transilluminator - Edas 290, using the 1-D 3.5 Kodak program.

Data analysis

As to the RAPD analysis, gels were interpreted for the presence or absence of bands, generating a binary matrix. In order to estimate genetic distances between lines, the complements of the Jaccard similarity coefficient were used. With regard to the codominant microsatellite marker (SSR), genetic distance was based on the frequency of alleles for each locus, using Rogers' modified distance (Goodman and Stuber, 1983). Wright's fixation coefficient F (Wright, 1965) was also calculated. Afterward, Pearson's correlation between distance matrices of RAPD and SSR markers was determined. Simplified representation of genetic distances between lines was obtained by UPGMA (unweighted pair-group method with arithmetic mean) and represented by a dendrogram. All analyses were performed using GENES (Cruz, 2006) and R (http://www.r-project.org) programs.

RESULTS AND DISCUSSION

The most appropriate amount of DNA for RAPD analysis was 15 ng, and $MgCl_2$ at a concentration of 2.5 mM produced well-separated and stained bands in the amplification reactions. These conditions were used for DNA amplification using nine RAPD primers. Through RAPD analysis, each primer produced bands comprising variable and easily detected intensities, as well as non-specific bands, which were discarded. The nine primers used (OPA03, OPA12, OPB07, OPB17, OPC05, OPC11, OPF05, OPL11, and OPM02) yielded 126 bands (Table 1). Of these, 104 were polymorphic (82.53%) and 22 monomorphic (17.47%). The lowest and highest number of polymorphic bands was 7 and 16 in the primers OPC05 and OPB17, respectively. The primer, which showed the least polymorphism, was OPC05, with 11 bands, of which 7 were polymorphic (63.64%). OPB17 showed the high-

Table 1. Number and frequency of polymorphic and monomorphic fragments obtained for each primer used to amplify the DNA of 10 popcorn lines.

Primers	Total number of fragments	Number of monomorphic fragments	(%)	Number of polymorphic fragments	(%)
OPA03	16	2	12.50	14	87.50
OPA12	16	4	25.00	12	75.00
OPB07	15	1	6.67	14	93.33
OPB17	19	3	15.79	16	84.21
OPC05	11	4	36.36	7	63.64
OPC11	10	2	20.00	8	80.00
OPF05	12	4	33.33	8	66.67
OPL11	11	1	9.09	10	90.91
OPM02	16	1	6.25	15	93.75
Total	126	22	17.46	104	82.54

est number of bands (19 of which 16 were polymorphic), and OPM02 yielded 16 bands and showed the largest number of polymorphic bands (93.75%). OPB07 produced only 15 bands, but was also considered highly polymorphic (14 polymorphic; 93.33%).

The level of polymorphism determined in this study is a little less than that obtained by Vilela et al. (2008) who detected 89.25% polymorphism. However, Vilela et al. (2008) tested 90 progeny of the open-pollinated popcorn variety, involving three recurrent selection cycles, where a greater variability is really expected for lines that show a narrow genetic base (Munhoz et al., 2009; Silva et al., 2009).

It is possible to infer that the RAPD markers were effective in detecting genetic variability between the lines evaluated in this study. P1-1 (Yellow Pearl I) and P9-3 (Avati Pichinga I) lines showed the highest values for genetic distance (0.4219), while P1-2 (Zélia I) and P1-3 (Curagua), and P1-2 (Zélia I) and P1-4 (Zélia II) revealed the lowest values for genetic distance (0.2394 and 0.2411, respectively). The greatest genetic distance between P1-1 and P9-3 can be explained by divergent genealogy, since Yellow Pearl I originated from a North-American composite, while Avati Pichinga has its origin in Paraguay. In turn, the common origin between Zélia I and Zélia II explains the higher genetic similarity found in the P1-1 and P1-2 lines. However, the high similarity between the P1-2 line, from the Zélia I variety (Pioneer triple hybrid), and line P1-3, formed from the Curagua variety (original from Chile), is unusual with regard to genealogical origin.

A cut performed at a distance of 0.32, considering the abrupt change-point in the dendrogram, allowed the composition of three groups: group I was constituted by P1-1 lines; group II included P9-1, P9-2, P9-3, P9-4, and P9-5, and group III clustered P1-2, P1-3, P1-4, and P1-5 lines (Figure 1). Placement of lines of populations P1 and P9 in distinct groups is evidence of RAPD marker consistency in the molecular characterization and analysis of popcorn, and is, therefore, in agreement with the purpose of establishing divergent lines in genetic breeding programs for the proper generation of divergent hybrids.

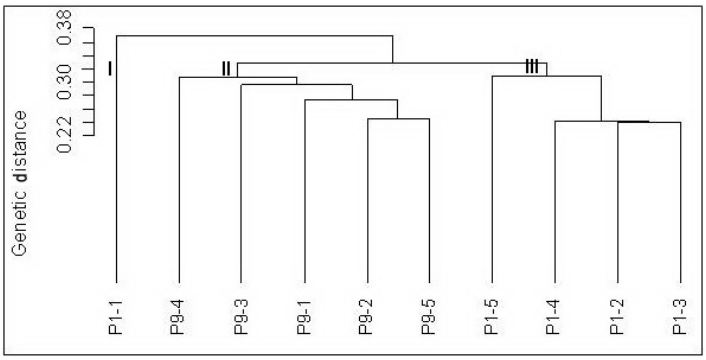

Figure 1. Genetic dissimilarity dendrogram organized using data obtained with RAPD markers for 10 popcorn lines evaluated (P1-1 to P1-5 and P9-1 to P9-5), determined by UPGMA analysis.

Lanza et al. (1997) found that RAPD markers were efficient for determining genetic diversity among maize lines, dividing them into different heterotic groups, and therefore, it was useful in the selection of superior lines for crossings, thus reducing the number of crossings for

evaluation in the field. In this context, Bruel et al. (2006) observed positive correlations between genetic divergences, detected by RAPD, and the averages determined in dialellic crossings, concerning the characteristics plant height, ear corn height, production, and seed weight. This corroborates the hypothesis that genetic divergence in lines is directly related to hybrid performance, emphasizing the efficiency of RAPD markers in the prediction of hybrid behavior.

According to the amplification of *SSR* loci, the number of alleles per microsatellite locus in the 10 popcorn lines ranged from 2 to 5, comprising a total of 47 alleles referring to the 14 primers used. The greatest number of alleles was found at the *Umc1653* and *Umc2281* loci, comprising 5 alleles, and at the *Umc2227*, *Umc1636* and *Umc2262* loci, comprising 4 alleles. The average number of alleles per locus was 3.36 (Table 2). Bracco et al. (2009), evaluating 131 popcorn landraces using 9 microsatellite loci, revealed a total of 65 alleles. On the other hand, Silva et al. (2009), evaluating 25 popcorn genotypes, observed that 23 microsatellites allowed the detection of 100 alleles.

Table 2. Sequence of microsatellite primers used in the estimation of genetic diversity in 10 popcorn lines, and number of alleles detected per primer and their position on chromosomes.

Locus	Nucleotide sequence	Number of alleles	Chromosome
Umc1071	GTGGTTGTCGAGTTCGTCGTATT (Reverse)	3	1
Umc1071	GTGGTTGTCGAGTTCGTCGTATT (Forward)		
Umc1336	CTCTGTTTTGGAAGAAGCTTTTGG (Reverse)	2	10
Umc1336	GTACAAATGATAAGCAAGGGGCAG (Forward)		
Umc1422	CTCATCGCGATCTCCCAGTC (Reverse)	3	2
Umc1422	GAGATAAGCTTCGCCCTGTACCTC (Forward)		
Umc1636	GTACTGGTACAGGTCGTCGCTCTT (Reverse)	4	9
Umc1636	CATATCAGTCGTTCGTCCAGCTAA (Forward)		
Umc1653	GCCGCCCACGTACATCTATC (Reverse)	5	6
Umc1653	GAGACATGGCAGACTCACTGACA (Forward)		
Umc2227	AGCTGAGCCTTCTCTTCTTGGCT (Reverse)	4	1
Umc2227	ACCTTGAGCGTGGAGTCGGT (Forward)		
Umc2245	CGTCGTCTTCGACATGTACTTCAC (Reverse)	3	2
Umc2245	GCCCTGTTATTGGAACAGTTTACG (Forward)		
Umc2262	CGTTCCCTGGTACCCTGTCTATAA (Reverse)	4	3
Umc2262	TCTGTTCGGGATTCTTCTTCAGTC (Forward)		
Umc2280	AAAAGAAGACGCTTTGTTTGTTGC (Reverse)	3	4
Umc2280	TTTTCGTCAACTTGATGTTTATGAGAGT (Forward)		
Umc2281	ATGATGATCTGCAGAGCCTAGTCC (Reverse)	5	4
Umc2281	CAATGATTGGAGCCTAACCCCT (Forward)		
Umc2292	ACTTCCGGCATGTCTTGTGTTT (Reverse)	3	5
Umc2292	AGCAGAAGAGGACAAACCAGATTC (Forward)		
Umc2293	ATGTTCCGTTTATTATTTGCCCG (Reverse)	3	5
Umc2293	AAAGAACAGACGCGATCCAATC (Forward)		
Umc2343	GACTGACAACTCAGATTTCACCCA (Reverse)	3	9
Umc2343	TCATCTTCCCCACAAATTTTCATT (Forward)		
Umc2350	CGAATCGAGGATGGTTTGTTTTT (Reverse)	2	10
Umc2350	AGTAGCGACTCCTCTGCGTGAG (Forward)		
Total		47	

Values for Wright's F-fixation coefficient (Wright, 1965) estimated based on frequency analysis of alleles on *SSR* loci showed that the heterozygous deficit in the 10 lines corresponded to 4.5% (F_{IS} = 0.045) and that the dissimilarities among the 10 lines considering the variation in the frequencies of the different alleles corresponded to 73.49% (F_{ST} = 0.7349). Therefore, there is high genetic divergence among the 10 popcorn lines evaluated.

Based on the frequency analysis of the 47 alleles on the *SSR* loci, genetic diversity was estimated by Rogers' (Goodman and Stuber, 1983) modified distance. The highest genetic distance (0.8495) was detected between P1-4 (Zélia II) and P9-3 (Avati Pichinga) lines, and the lowest distance (0.2092), between P1-2 (Zélia I) and P1-4 (Zélia II). The highest distance between Zélia II and Avati Pichinga lines based on the analysis of *SSR* loci can also be explained by geographic distance of the varieties developed in Brazil and in Paraguay and by the different soil and climate adaptations.

Through a cut performed in the dendrogram at a distance of 0.45, considering the abrupt change-point, it was possible to form two groups. Group I comprised the lines P9-1, P9-2, P9-3, P9-4, and P9-5 and group II the P1-1, P1-2, P1-3, P1-4, and P1-5 lines (Figure 2). The comparison of the groups formed using RAPD and SSR markers revealed similarities, showing that, in spite of the fact that some information about the SSRs corresponds to molecular markers containing a greater amount of information than with RAPD analysis (Demeke et al., 1997; Schlötterer, 2004; Schulman, 2007), such data cannot be conceived in an unconditional way.

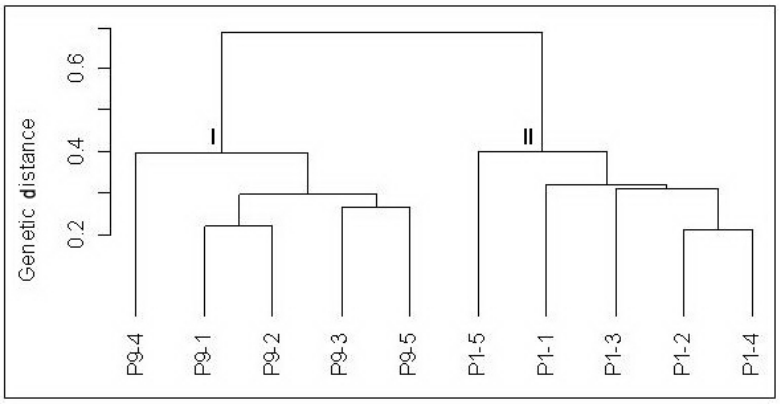

Figure 2. Genetic dissimilarity dendrogram organized using data obtained through microsatellite markers for 10 popcorn lines evaluated (P1-1 to P1-5 and P9-1 to P9-5), defined by UPGMA grouping method.

Estimates of correlation between genetic distances obtained by RAPD and SSR markers were moderate, with magnitudes of 0.5453, indicating that there is a relative pattern of association between results obtained using these two analytical procedures in order to obtain markers of DNA fragments (Figure 3). This result is in accordance with other studies carried out using maize, such as those of Pejic et al. (1998) and Souza et al. (2008), who found correlations of 0.57 and 0.54, respectively, between RAPD and SSR markers. Nevertheless, there are studies that showed a low correlation between RAPD and SSR markers, such as the research of Garcia et al. (2004) who observed a correlation of 0.33. According to Lu and Bernardo (2001), the inconsistency of results obtained using different markers, mainly to evaluate different lines, can be explained by the fact that molecular markers evaluate different components of DNA variation, and that they can progress in diverse ways. Yet, despite these explanations, the fact is that the lack of consistency observed between RAPD markers and *SSR* locus analysis has discouraged RAPD use, and in an implicit way has cast doubts as to the efficacy of the technique as well as to skepticism about information obtained through RAPD analysis.

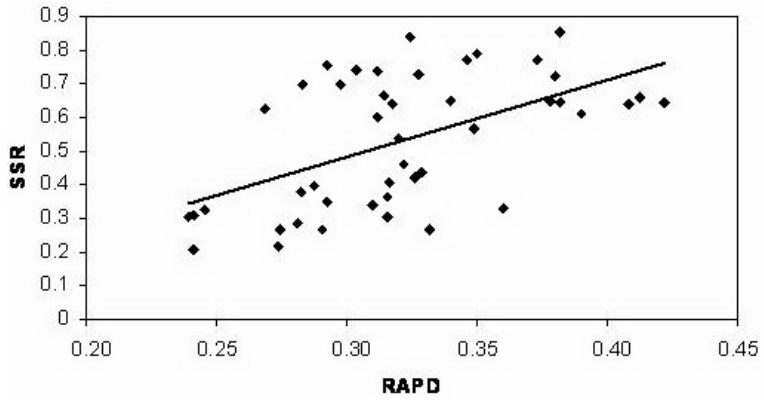

Figure 3. Dispersion diagram (graph) organized according to the genetic distances, using SSR and RAPD markers. The straight line shows the degree of correlation between genetic distances. R^2 is the coefficient of determination for straight line fit.

Our analysis using popcorn lines showed that both techniques may provide consistent data, and can thereby be used to study genetic diversity in popcorn, showing concordant values of genetic diversity. This led us to conclude that RAPD markers can be considered as effective as *SSR* locus markers when there is a greater amount of investment to: i) obtain DNA of high purity after the extraction process; ii) select RAPD primers aimed at identifying DNA segments that are well separated and reproducible, iii) standardize reagent concentrations that are critical in the amplification process, providing reliable and replicable results, and iv) identify DNA segments more strongly stained, selecting properly different amplification programs. Therefore, the option to analyze popcorn genetic diversity using RAPD markers to keep track and guide genetic breeding programs can be considered an adequate strategy. On practical grounds, the OPB17, OPM02 and OPB07 primers, for instance, which produced the greatest number of bands and which showed the greatest potential to discriminate polymorphic DNA segments, can be recommended for future analysis of the popcorn genome using RAPD markers.

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REFERENCES

Aguiar CG, Schuster I, Amaral AT Jr, Scapim CA, et al. (2008). Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. *Genet. Mol. Res.* 7: 1233-1244.

Amaral Júnior AT (1999a). Divergência genética entre acessos de moranga do banco de germoplasma de hortaliças da Universidade Federal de Viçosa. *Hortic. Bras.* 17: 3-6.

Amaral Júnior AT, Casali VWD, Cruz CD and Finger FL (1999b). Genetic inferences on yield and quality of tomato in a diallel cross. [Inferências genéticas na produção e qualidade de tomateiro sob cruzamento dialélico]. *Pesq. Agropec. Bras.* 34: 1407-1416.

Bernardo R (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Sci.* 48: 1649-1664.

Binneck E, Nedel JL and Dellagostin OA (2002). RAPD analysis on cultivar identification: a useful methodology? [Análise de RAPD na identificação de cultivares: uma metodologia útil?]. *Rev. Bras. Sem.* 24: 183-196.

Bracco M, Lia VV, Gottlieb AM, Camara HJ, et al. (2009). Genetic diversity in maize landraces from indigenous settlements of Northeastern Argentina. *Genetica* 135: 39-49.

Bruel DC, Carpentieri-Pípolo V, Gerage AC, Fonseca Júnior NS, et al. (2006). Genetic distance estimated by RAPD markers and its relationship with hybrid performance in maize. *Pesq. Agropec. Bras.* 41: 1491-1498.

Chen X and Sullivan PF (2003). Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. *Pharmacogenomics J.* 3: 77-96.

Cruz CD (2006). Programa GENES: Estatística Experimental e Matrizes. Universidade Federal de Viçosa, Viçosa.

Dandolini TS, Scapim CA, Amaral Júnior AT, Mangolin CA, et al. (2008). Genetic divergence in popcorn lines detected by microsatellite markers. *Crop Breed. Appl. Biotechnol.* 8: 313-320.

Demeke T, Sasikumar B, Hucl P and Chibbar RN (1997). Random amplified polymorphic DNA (RAPD) in cereal improvement. *Maydica* 42: 133-142.

Don RH, Cox PT, Wainwright BJ, Baker K, et al. (1991). 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.* 19: 4008.

Garcia AAF, Benchimol LL, Barbosa AMM, Geraldi IO, et al. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genet. Mol. Biol.* 27: 579-588.

Goncalves LS, Rodrigues R, Amaral AT Jr, Karasawa M, et al. (2008a). Comparison of multivariate statistical algorithms to cluster tomato heirloom accessions. *Genet. Mol. Res.* 7: 1289-1297.

Gonçalves LSA, Sudré CP, Bento CS, Moulin MM, et al. (2008b). Divergência genética em tomate estimada por marcadores RAPD em comparação com descritores multicategóricos. *Hortic. Bras.* 26: 364-370.

Gonçalves LS, Rodrigues R, do Amaral Júnior AT, Karasawa M, et al. (2009). Heirloom tomato gene bank: assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. *Genet. Mol. Res.* 8: 364-374.

Goodman MM and Stuber CW (1983). Races of maize. VI. Isozyme variation among races of maize in Bolivia. *Maydica* 28: 169-187.

Hoisington D, Khairallah M and González-Léon D (1994). Laboratory Potocols: CIMMYT Applied Molecular Genetics Laboratory. 2nd edn. CIMMYT, Mexico.

Kantety RV, Zeng XP, Bebbetzen JL and Zehr BE (1995). Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbreed lines using intersimple sequence repeat (ISSR) amplification. *Mol. Breed.* 1: 365-373.

Kilian A, Huttner E, Wenzl P, Jaccoud D, et al. (2005). The Fast and the Cheap: SNP and DArT-based Whole Genome Profiling for Crop Improvement. In: Proceedings of the International Congress in the Wake of the Double Helix: From the Green Revolution to the Gene Revolution (Tuberosa R, Phillips RL and Gale M, eds.). May 27-31, Bologna, 443-461.

Lanza LLB, Souza Júnior CL, Ottoboni LMM, Vieira MLC, et al. (1997). Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. *Theor. Appl. Genet.* 94: 1023-1030.

Li YL, LV DB, Wang YZ, Chen SJ, et al. (2004). Study on the genetic diversity of popcorn inbreds and their germplasm relationship with normal corn inbreds using SSR markers. *Maydica* 49: 327-333.

Lu H and Bernardo R (2001). Molecular marker diversity among current and historical maize inbreds. *Theor. Appl. Genet.* 103: 613-617.

Mohammadi SA and Prasanna BM (2003). Analysis of genetic diversity in crop plants - salient statistical tools and considerations. *Crop Sci.* 43: 1235-1248.

Moose SP and Mumm RH (2008). Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiol.* 147: 969-977.

Mullis KB and Faloona FA (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155: 335-350.

Munhoz REF, Prioli AJ, Amaral Júnior AT, Scapim CA, et al. (2009). Genetic distances between popcorn populations based on molecular markers and correlations with heterosis estimates made by diallel analysis of hybrids. *Genet. Mol. Res.* 8: 951-962.

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, et al. (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theor. Appl. Genet.* 97: 1248-1255.

Saiki RK, Scharf S, Faloona F, Mullis KB, et al. (1985). Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354.

Saker MM, Youssef SS, Abdallah NA, Bashandy HS, et al. (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *Afr. J. Biotechnol.* 4: 882-890.

Santacruz-Varela A, Widrlechner MP, Ziegler KE, Salvador RJ, et al. (2004). Phylogenetic relationships among North

American popcorns and their evolutionary links to Mexican and South American popcorns. *Crop Sci.* 44: 1456-1467.
Schlötterer C (2004). The evolution of molecular markers - just a matter of fashion? *Nat. Rev. Genet.* 5: 63-69.
Schulman AH (2007). Molecular markers to assess genetic diversity. *Euphytica* 158: 313-321.
Silva TA, Pinto RJB, Scapim CA, Mangolin CA, et al. (2009). Genetic divergence in popcorn genotypes using microsatellites in bulk genomic DNA. *Crop Breed. Appl. Genet.* 9: 31-36.
Souza SGH, Carpentieri-Pípolo V, Ruas CF, Carvalho VP, et al. (2008). Comparative analysis of genetic diversity among the maize inbred lines (*Zea mays* L.) obtained by RAPD and SSR markers. *Braz. Arch. Biol. Technol.* 51: 183-192.
Sudré CP, Leonardecz E, Rodrigues R, Amaral Júnior AT, et al. (2007). Genetic resources of vegetable crops: a survey in the Brazilian germplasm collections pictured through papers published in the journals of the Brazilian Society for Horticultural Science. *Hortic. Bras.* 25: 496-503.
Tautz D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17: 6463-6471.
Vilela FO, Amaral Júnior AT, Pereira MG, Scapim CA, et al. (2008). Effect of recurrent selection on the genetic variability of the UNB-2U popcorn population using RAPD markers. *Acta Sci. Agron.* 30: 25-30.
Vos P, Hogers R, Bleeker M, Reijans M, et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407-4414.
Welsh J and McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213-7218.
Williams JG, Kubelik AR, Livak KJ, Rafalski JA, et al. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.
Wright S (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395-420.
Ziegler KE and Ashman B (1994). Popcorn. In: Specialty Corns (Hallauer AR, ed.). CRC Press, Iowa, 189-223.
Zietkiewicz E, Rafalski A and Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.